Embryonic Development from First Cleavage Through Seventy-Two Hours Incubation in Two Strains of Pekin Duck (*Anas platyrhynchos*)

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ABSTRACT Embryonic mortality is a significant problem plaguing the commercial duck industry worldwide. Yet, an objective means to stage development of the duck embryo is lacking. Such a staging procedure, which is described in this study, is essential for the critical and reproducible assessment of embryo development. The morphological features associated with duck embryo development are very similar to those of the chicken, although the duck embryo develops more slowly. The staging scheme presented here provides objective morphological criteria describing the embryonic development of the duck.

(*Key words*: duck, embryogenesis, blastoderm, avian)

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INTRODUCTION

Despite the fact that early embryonic mortality is high in poultry (Coleman, 1983; Krueger, 1990), the biological basis of early embryonic mortality is not known. In poultry, the first cleavage furrow appears about 6 to 8 h after fertilization (Perry, 1987). However, due to the difficulties of egg mass extraction from the anterior end of the oviduct without euthanizing the hen, the study of early embryonic development has long been limited to embryos in laid eggs (Foulkes, 1990). In fact, most descriptions of embryo development start with the emergence of the primitive streak (6 to 8 h of incubation in the chicken), which marks the beginning of gastrulation.

Hamburger and Hamilton [HH; (1951)] formulated the numerical staging of normal development of the postoviposition chick embryo that is most commonly used today. The HH staging procedure consists of 46 stages that can be summarized as three periods: 1) appearance and extension of the primitive streak up to cephalic fold emergence (Stages HH 2 to 6), 2) appearance and multiplication of the somites up to embryonic flexion emergence (Stages 7 to 14), and 3) embryonic organogenesis and development of the extra-embryonic structures (Stages 15 to 46). Hamburger and Hamilton (1951) classified the whole oviductal period of embryonic growth and the first hours of postoviposition/incubation (up to primitive streak emergence) as Stage 1 (HH). Eyal-Giladi and Kochav [EGK; (1976)] were the first to provide a descriptive sequence of chicken embryo development throughout the oviductal period, that is, from fertilization through oviposition. Later, Gupta and Bakst [GB; (1993)] and Bakst et al. (1997) established the normal sequence of embryonic development in the turkey embryo for the same period. In general, this period of early embryonic development in the chicken and turkey covers three phases: 1) cleavage period (in the oviduct), which is a period of intense cell division [Stages (EGK) I to VI; Stages (GB) I to VI]; 2) formation of the area pellucida (in the oviduct), which consists of the thinning of the central zone of the blastodisc as a consequence of cell shedding [Stages (EGK) VII to IX; Stages (GB) VII to VIII]; and 3) formation of the hypoblast (first 6 h of incubation in the chicken), which results from the ingression of cells from the ventral surface of the embryo and the migration of cells from the marginal zone [Stages (EGK) X to XIV; Stages (GB) IX to XI].

The only description of the normal sequence of duck embryonic development was done with the Pekin duck (Kaltofen, 1971). However, Kaltofen (1971) does not describe embryonic development in the oviduct and is not very precise in the descriptions given that the interval between two consecutive stages is 24 h. Two other investigators have described embryonic development of the Pekin duck more precisely in the first hours of egg incubation. Pasteels (1945) noted that the area pellucida and

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Abbreviation Key: dStage = duck stage; EGK = Eyal-Giladi and Kochav; GB = Gupta and Bakst; HH = Hamburger and Hamilton; PL = perivitelline layer.

hypoblast (here called primary entoderm) formation leads to the bilaminar embryo (presumably the epiblast and hypoblast). The period described covered the first 16.5 h of incubation. Chen (1932) described the morphological development of the duck embryo between egg laying and the 1 to 3 somite stage, which covers the first 35 h of incubation. However, both sets of descriptions commenced at oviposition and ignored the oviductal period of development. Therefore, the objective of this study was to describe the oviductal phase of embryonic development of the duck, with emphasis on the period between fertilization and the 72 h of incubation, a period characterized by relatively high embryonic death in the Pekin duck (Dupuy, personal observation). By using the staging procedure, we also compared the rates of embryo mortality between two commercial strains of ducks.

MATERIALS AND METHODS

Embryos from Oviductal Eggs

The collection of oviductal eggs for staging embryo development prior to oviposition was performed at Maple Leaf Farms Inc.,[2] with 500 Pekin duck breeders. Two hundred fifty hens from each of two strains (A and B), characterized by hatchability differences, were used at peak of lay. At the time of this study, hatchability of Strain A was 83%, and Strain B was 73%. Hatchability here is defined as the number of ducklings hatched divided by the total number of eggs set. All breeders were 63 wk old. The ducks were raised separately on litter (wood shavings) and exposed to 18 h light and 6 h dark per day. Natural mating was used with a ratio of one drake per five females. Ducks from Strains A and B were mated with Strain C males.

Ducks were euthanized by cervical dislocation 3, 6, 9, 12, 15, 18, 21, and 24 h after the previous oviposition (±1 h), and the oviductal egg mass removed. About 30 embryos per period were evaluated. In addition, 40 embryos per line from freshly laid eggs (removed within 15 min of oviposition) were also evaluated.

Embryo Collection and Classification. The eggshell was opened and the albumen was removed. The perivitelline layer (PL) was cut around the embryo. The embryo with surrounding PL and yolk was collected with a spoon and placed in a Petri dish containing phosphate buffer saline, pH 7.4, and cleared of yolk. An attempt was made to stage each embryo according to the methods of Eyal-Giladi and Kochav (1976) and Gupta and Bakst (1993). The embryos were then fixed in a PBS solution containing 4% paraformaldehyde and 1% glutaraldehyde.

Eggshell Thickness. To determine the relationship between stage of embryonic development and eggshell thickness, portions of the eggshell plus shell membrane were randomly collected from each egg. Thickness was measured with a digital micrometer. Six measurements were made for each egg.

Embryos from Laid Eggs

Eggs and Incubation. A total of 1,560 eggs was set; equal numbers of Strain A and Strain B were used. As above, both strains of Pekin ducks had been mated with drakes from Strain C. Eggs were collected within 3 h after oviposition, washed, and sanitized at the Maple Leaf Farm hatchery and were shipped overnight to Beltsville, MD. At Beltsville, all broken eggs were discarded, and the others were weighed within 2 h of delivery. Incubation was performed at 37.6 C with a relative humidity of 60%. Embryos were evaluated within 2 h after removal from the incubator. All eggs were weighed just after incubation and the percentage of egg weight loss determined.

Nine equal groups of eggs were examined immediately after weighing with no prior incubation or after cold storage for 24 h at 16 to 17 C. They were then incubated 3, 6, 9, 12, 15, 18, 21, and 24 h, respectively. In this phase of the study, Strain A ducks were 55 to 59 wk old, and Strain B ducks were 37 to 44 wk old. Eggs from Strains A and B, which were 55 to 65 and 51 to 58 wk old, respectively, were incubated 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 66, 69, and 72 h.

Embryo Collection and Staging. Embryos were isolated and eggshell thickness was determined as described for oviductal eggs. For embryos beyond hypoblast formation, the HH staging procedure was used. After classification, the embryos were fixed in 4% paraformaldehyde (vol/vol) and 1% glutaraldehyde (vol/vol) in PBS.

Statistical Analyses. Fertility, based on visual inspection as well as embryonic mortality and embryonic abnormalities, was analyzed. The strain (A or B) effect on fertility, mortality, and embryonic abnormalities was analyzed by a CATMOD procedure on SAS® software (SAS Institute, 1988), which uses the general linear model (GLM) applied to categorical data. A probability level of $P < 0.05$ was considered significant.

RESULTS

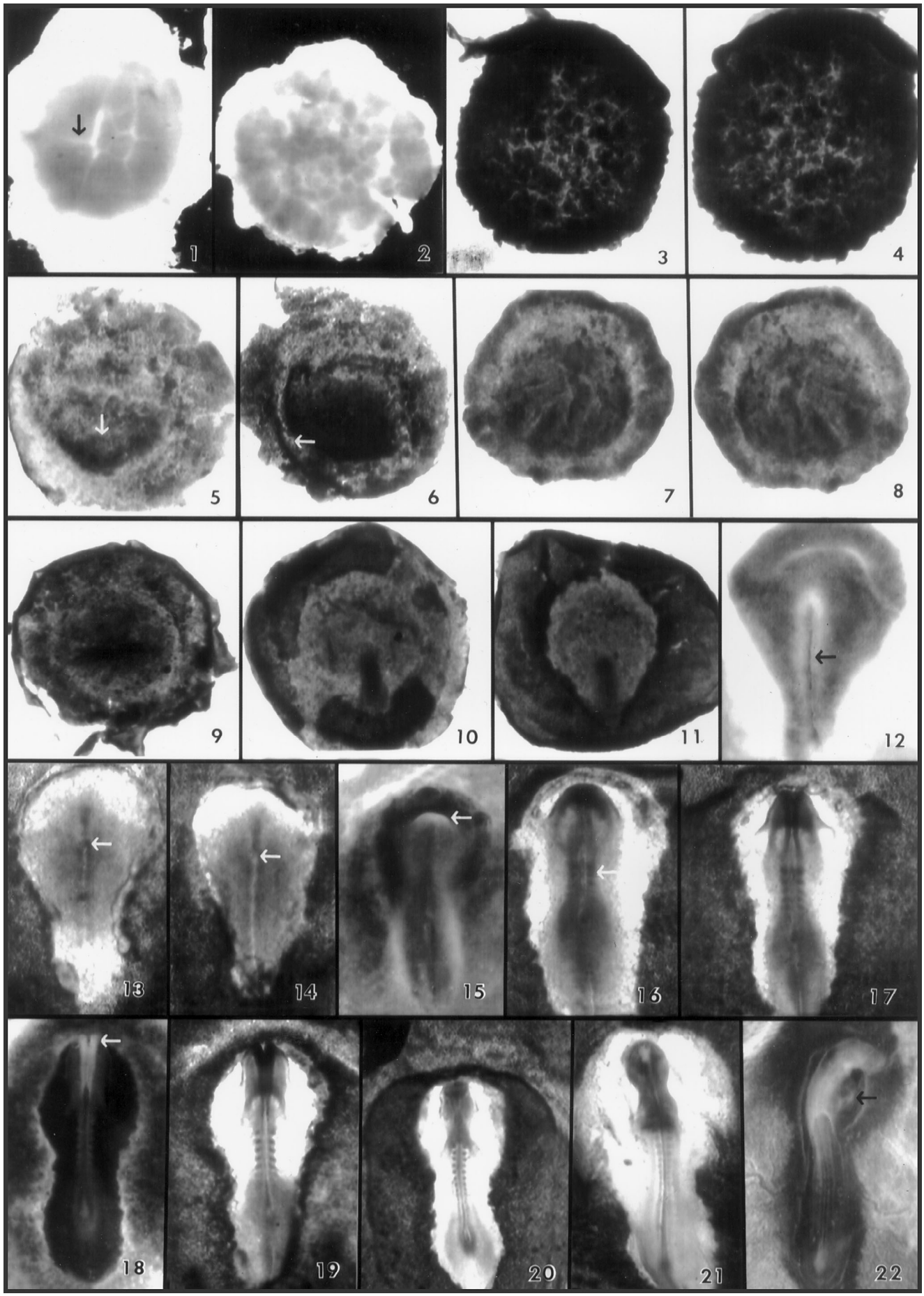
Duck Embryo Staging

Development of the Pekin duck embryo between first cleavage and 72 h of incubation was divided into the following phases: cleavage phase (Stages 1 to 6), area pellucida formation (Stages 7 to 9), hypoblast formation (Stages 10 to 14), primitive streak (Stages 15 to 17), somite (Stages 18 to 27), and organogenesis (Stages 28 to 33). The morphological characteristics defining each stage are presented below with the corresponding stage for the pregastrulation chicken (EGK) and turkey (GB) embryo and the postgastrulation chicken embryo (HH). (Not all stages are shown in the figures.)

Cleavage Phase

Stage 1 (EGK I; GB I; Figure 1). Asymmetrical cleavage furrows can be observed on the dorsal side of the embryo.

[2]Milford, IN.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Stage 2 (EGK II; GB II; Figure 2). In the center of the dorsal side, a cluster of several closed cells can be observed. These are surrounded by large open cells separated by cleavage furrows.

Stage 3 (EGK III; GB III). The number of closed cells in the center of the embryo has increased from 100 to 150 on the dorsal side, and the large open cells are relegated toward the periphery. On the ventral side, some cleavage furrows are visible.

Stage 4 (EGK IV; GB IV). On the dorsal side, about 300 closed cells are visible in the center, whereas the peripheral open cells are not as numerous. On the ventral side, several dozen of closed cells are visible. A tiny cavity (the subgerminal cavity) appears between the embryo proper and the yolk.

Stage 5 (EGK V; GB V). The area of closed cells extends nearly to the edge of the embryo on the dorsal and ventral sides. Some spherical cells remain on the dorsal surface of the embryo. On the ventral side of the embryo, the diameter of subgerminal cavity is at least half of the embryo diameter.

Stage 6 (EGK VI; GB VI). At this stage, cells on the dorsal side are uniform and are discernible only with a microscope. Cells on the ventral side are large and appear to be similar to the yolk-laden cells observed in Stage EGK VIII for the chicken. The subgerminal cavity has extended, and the embryo is now referred to as the blastoderm.

Area Pellucida Formation

Stage 7 (EGK VII, GB VII: oviposition in turkey). Shedding of cells from the ventral side of the blastoderm is first observed at what will be the posterior end of the embryo. This phenomenon is the first sign of the appearance of a translucent zone in the center of the embryo, the area pellucida.

Stage 8 (EGK VIII; GB VII). The translucent zone, the area pellucida, covers a large part of the embryo, particularly in the posterior region. A peripheral zone, the area opaca, is not affected by the cell shedding process, and its appearance does not change during area pellucida formation.

Stage 9 (EGK IX; GB VIII). The area pellucida extends towards the anterior part of the blastoderm. The boundary between the area pellucida and the area opaca becomes more distinct, especially in the posterior part of the embryo.

Hypoblast Formation

Stage 10 (EGK X, oviposition in chicken; GB IX; Figures 3 and 4). At oviposition, the area pellucida is

FIGURE 1. Asymmetrical cleavage furrows can be observed on the dorsal side of this duck Stage1 embryo. The arrow highlights a cleavage furrow separating two peripheral "open" cells.

FIGURE 2. A cluster of several closed cells can be observed in the center of the dorsal face of this duck Stage 2 embryo.

FIGURE 3 (dorsal surface) and FIGURE 4 (ventral surface). At oviposition (duck Stage 10) small groups of cells are apparent in the area pellucida, which is easily distinguishable from the uniformly dense peripheral area opaca.

FIGURE 5. Although the first evidence of hypoblast formation may appear at duck Stage 10, definitive hypoblast is observed at duck Stage 11. An arc-shaped thickening (arrow) marks the posterior limit of the hypoblast and the future posterior end of the embryo.

FIGURE 6. At duck Stage 12, the hypoblast has extended in an anterior direction and has a sheet-like appearance, due to the fusion of the diffuse arc and some cell groups. In this figure, the hypoblast covers more than half of the surface area of the area pellucida. The marginal zone (arrow) is clearly visible between the dense area of the hypoblast and the area opaca.

FIGURE 7 (dorsal surface) and FIGURE 8 (ventral surface). In this late duck Stage 12 embryo, the process of hypoblast formation is nearly completed. Except for the peripheral marginal zone, the hypoblast fills the area of the area pellucida.

FIGURE 9. This duck Stage 13 embryo possesses a completed hypoblast. A defined marginal zone and area opaca are also observed.

FIGURE 10. In the posterior end of this duck Stage 15 embryo between the posterior part of the hypoblast and the area opaca is an elongated, cone shaped primitive steak.

FIGURE 11. In this late duck Stage 15 embryo, the primitive streak appears as a dense bulge and the area pellucida is beginning to assume a pear-shaped profile.

FIGURE 12. In this early duck Stage 17 embryo, the area pellucida has a pear-shaped profile, the small end of which is at the posterior part of the embryo. A furrow, the primitive groove (arrow) is visible along the primitive streak. Hensen's node is just visible at the anterior end of the primitive streak.

FIGURES 13 and 14. In these duck Stage 17 embryos, the arrow indicates Hensen's node. Extending anteriorly from Hensen's node is the notochord and posteriorly is the primitive streak.

FIGURE 15. Although not clearly visible in this duck Stage 18 embryo, the notochord extends from the region of the Hensen's node towards the anterior of the embryo. At the anterior end of this thin stick-shaped structure, a tiny convex extension of the neural plate is visible. This is the head-fold (arrow).

FIGURE 16. At duck Stage 20, one or two pairs of somites are visible (arrow). Neural folds can be observed in the cephalic region on each side of the neural plate.

FIGURE 17. An early duck Stage 21 embryo is observed with five somites.

FIGURE 18. In this duck Stage 21 embryo, five pairs of somites are visible and the neural folds appear to be merging in the cephalic region (arrow). In addition, the posterior zone of the notochord is surrounded by the posterior portion of the neural folds forming the sinus rhomboidalis.

FIGURE 19. Another view of a duck Stage 21 embryo highlighting the cranial region.

FIGURE 20. In this duck Stage 23 embryo, nine pairs of somites are visible.

FIGURE 21. In this duck Stage 24, 13 pairs of somites are visible and there is a slight head flexure towards the left. The anterior part of the neural folds have started to merge, which is the first sign of the neuropore closure at the anterior end of the neural tube.

FIGURE 22. In this duck Stage 27 embryo, 23 pairs of somites were visible by careful examination. The head flexure is now much more pronounced with the axes of the anterior brain and the posterior brain forming a right angle. The arrow highlights the ventricular loop of the heart.

easily distinguished from the area opaca. The first signs of hypoblast formation may be visible at the ventral side of the area pellucida. Small groups of cells emerge and form a reticular structure. The marginal zone of the area pellucida (next to the area opaca border) remains translucent and does not contain cell groups.

Stage 11 (EGK XI; GB IX; Figure 5). Although the first evidence of hypoblast formation may appear at Stage 10, a definitive hypoblast is observed at Stage 11. A diffuse arc-shaped structure marks the posterior limit of the hypoblast and the future posterior end of the embryo. Small groups of cells are also visible on the anterior portion of the ventral side.

Stage 12 (EGK XII; GB X; Figures 6 to 8). At this stage the hypoblast has extended in an anterior direction and has a sheet-like appearance, due to the fusion of the diffuse arc and some cell groups. The hypoblast covers the posterior half of the area pellucida except for the marginal zone. Small cell groups remain visible on the anterior portion of the blastoderm.

Stage 13 (EGK XIII; GB XI; Figure 9). The process of hypoblast formation is now completed. The embryo has a two-layer structure. The upper layer, or epiblast, forms the dorsal part of the embryo. The lower layer, or hypoblast, covers the central part of the ventral side of the area pellucida.

Stage 14 (EGK XIV). A cellular bridge forms on the ventral side of the blastoderm between the posterior part of the hypoblast and the area opaca. This bridge may precede the emergence of the primitive streak. However, in ducks, this cellular bridge was apparent in about 25% of the embryos, which suggests that this is a rapid transitional phase.

Primitive Streak Phase

Stage 15 (HH 2; Figures 10, 11). The primitive streak appears as a cone-shaped bulge in the region of the cellular bridge.

Stage 16 (HH 3). The primitive streak extends anteriorly, reaches approximately the middle of area pellucida, and appears as a dense bulge.

Stage 17 (HH 4; Figures 12 to 14). The area pellucida has a pear-shaped profile, the small end of which is at the posterior part of the embryo. The length of the primitive streak is maximal covering about two-thirds of the area pellucida. A furrow (primitive groove) is visible along the primitive streak. At the anterior end of the primitive groove, a tiny depression is visible, the primitive pit. The primitive pit is surrounded by a large, thick structure (Hensen's node).

Somite Phase

Stage 18 (HH 5; Figure 15). The notochord extends from the region of the Hensen's node towards the anterior end of the embryo. At the anterior end of this thin stick-shaped structure, a tiny convex extension of the neural plate is visible. This extension is the head-fold.

Stage 19 (HH 6). The head-fold forms an indentation on the dorsal side of the embryo. The primitive streak bends in the Hensen's node region. Around this curve, two thicker structures define the Hensen's node. No somite is visible.

Stage 20 (HH 7; Figure 16). Between Stage 20 and Stage 27, the simplest way to classify the embryos is to count the number of somites. At Stage 20, one or two pairs of somites are visible. Neural folds can be observed in the cephalic region on each side of the neural plate.

Stage 21 (HH 8; Figure 17). Three to five pairs of somites are visible. The neural folds merge in the middle of the cephalic region. When five pairs of somites are visible, the posterior zone of the chord is surrounded by the posterior portion of the neural folds and forms the sinus rhomboidalis. Blood islands are visible on the posterior end of the embryonic mass.

Stage 22 (HH 9). Six to eight pairs of somites are visible. The primary optic vesicles appear. The heart primordia visible on each side of the central axis begins to merge.

Stage 23 (HH 10; Figure 20). Nine to 11 pairs of somites are visible. Three brain vesicles are clearly visible. The optic vesicles have grown in volume but they still do not have a constriction in their base. The heart bends slightly to the right side.

Stage 24 (HH 11; Figure 21). Twelve to 14 pairs of somites are visible. A slight head flexure towards the left appears. The anterior part of the neural folds begins to merge, which is the first sign of the neuropore closure. Five units (or neuromeres) are visible on the posterior brain. The optic vesicles are constricted in their base. The heart bending to the right is more pronounced.

Stage 25 (HH 12). Fifteen to 17 pairs of somites are visible. The head of the embryo starts to bend to the left. The neuropore is closed. The anterior part of the head (telencephalon) becomes prominent. The optic vesicles are now well formed. The heart is slightly S-shaped. Blood vessels are visible in the area vasculosa surrounding the embryo proper. The amniotic fold covers the anterior brain.

Stage 26 (HH 13). Eighteen to 20 pairs of somites are visible. The head is partly bent to the left and the telencephalon has completed development. The atrio-ventricular canal of the heart is defined by a constriction and blood vessels form a complete circuit that covers the embryo and the area vasculosa. The amniotic fold covers the anterior brain, the middle brain, and the anterior part of the posterior brain.

Stage 27 (HH 14; Figure 22). Twenty-one to 23 pairs of somites are visible. The head flexure is now much more pronounced with the axes of the anterior brain and the posterior brain forming a right angle. The body flexure reaches somites 7 to 9. The primitive optic vesicles begin to invaginate and the crystalline lens buds start to form. In the pharynx region, visceral arches 1 and 2 and visceral clefts 1 and 2 are distinct. The posterior arches are still not visible. The amnion extends up to somites 7 to 10. After Stage 27, the number of somites is difficult to count accurately. That is why the morphology of limbs, visceral

arches, and other more obvious body structures are used to classify the embryos from Stages 28 to 33.

Organogenesis Phase

Stage 28 (HH 15). The head flexure is more pronounced and the axes of the anterior brain and the posterior brain form an acute angle. The body flexure extends to somites 11 to 13. Visceral arch 3 and the visceral cleft 3 are distinct. The optic cupule is formed. The double outline of the iris is visible. The amnion extends to somites 7 to 14. The zone of emergence of the limb buds is still flat and unmarked. The lateral body folds reach somites 15 to 17.

Stage 29 (HH 16). The flexures are more pronounced than in Stage 28 and constrictions between the different parts of the brain are distinct. Visceral cleft 3 is oval-shaped. The amnion extends to somites 10 to 18. The wing buds form a fine crest whereas the leg buds are still invisible. The tail bud forms a short and straight cone. The lateral body folds extend behind the wing buds to somites 17 to 20.

Stage 30 (HH 17). The head and body flexures are more pronounced. The general rotation of the body extends up to somites 17 or 18. The amniotic extension is highly variable, but the embryo body is never totally covered at this stage. The wing and leg buds are small bulges of equivalent size. The tail bud bends to the right. The lateral body folds surround the embryo body. The nasal pits are clearly visible.

Stage 31 (HH 18). In the head flexure region, the medulla axis forms approximately a right angle with the trunk axis. The body flexure extends up to the lumbar region. An oval-shaped amniotic hole is visible in the lumbar region. The leg buds are generally slightly longer than the wing buds and the tail bud has bent to the left forming a right angle with the body axis. The allantois emerges as a thick-walled pocket not yet vesicular.

Stage 32 (HH 19). In the head flexure region, the medulla axis forms an acute angle with the trunk axis. The whole body is in rotation. The amnion may be closed at this stage, but most of the embryos still have a lumbar amniotic hole. The limb and tail buds are longer than in Stage 31 and they are symmetrical. The legs are slightly longer and thicker than the wings. The tail bud is bent with the end towards the anterior part of the embryo. The maxilla bud consists of a distinct bulge. The visceral arch 4 presents a distinct groove on its dorsal side and a superficial furrow on its ventral side. The allantois is a pocket of variable size not yet vesicular. The eyes are still not pigmented.

Stage 33 (HH 20). The head flexure extends further and the body rotation is completed. The amnion is usually closed, but some embryos still present a hole in the lumbar region. The leg buds are slightly asymmetrical and are longer than the wing buds. The maxilla bud is now clearly visible. The allantois is a vesicular pocket of variable size. The eyes are slightly grayish pigmented.

Fertility, Embryonic Mortality, Embryonic Abnormalities

Oviductal Eggs. Strain B had significantly lower true fertility (91.4 vs. 99.2%; $P < 0.001$) and significantly higher embryonic mortality (5.5 vs. 1.5%; $P < 0.05$) than Strain A (Table 1). Embryonic abnormality rates were not significantly different between strains. Mortality was most prevalent during duck Stage (dStage) 2.

Fertilization Through 24 h of Incubation. These data support the observations observed with oviductal egg embryos. Strain B had significantly lower fertility (87.9 vs. 98.9%; $P < 0.001$) and significantly higher embryonic mortality (5.8 vs. 0.4%; $P < 0.01$) than Strain A (Table 1). No significant differences in embryonic abnormality rates were observed between strains. Embryonic death was most prevalent during dStages 1 to 3 and dStages 5 to 6.

Fertilization Through 72 h of Incubation. The results of the statistical analysis of fertility, embryonic mortality, and embryonic abnormalities after 72 h of incubation are shown in Table 1. Strain B had significantly lower fertility (78.4 vs. 97.4%; $P < 0.001$) and significantly higher embryonic mortality (4.3 vs. 1.8%; $P < 0.05$) than Strain A. Embryos appear to die around dStage 2 and within the primitive streak stage (dStages 16 to 19) in Strain A and around the emergence of the head-fold and the first somites (dStages 19 to 22) in Strain B.

DISCUSSION

The development of the Pekin duck embryo is morphologically similar to that of the chicken embryo as described by Eyal-Giladi and Kochav (1976) and Hamburger and Hamilton (1951). However, instead of superimposing their number system on the duck stages described, we used a continuously numbered succession from first cleavage through hatching.

Based on our observations, hatchability differences found between Strains A and B at the hatchery are predictable even before oviposition and become more pronounced with incubation. These strain differences in hatchability do not seem to be due to a male effect, as the males used in the two flocks are from the same strain, same age and used under the same conditions. Furthermore, the environmental conditions at the duck farms were as standardized as possible and therefore unlikely to be the cause of strain hatchability and fertility differences.

Cleavage Period

The first cleavage (dStage 1) is visible in most duck embryos as soon as the third hour after the previous oviposition, even before the shell membrane is formed. The first cleavage furrows could be observed in the magnum, whereas they appear only in the isthmus in quail (Stepinska and Olszanska (1983) and in the uterus in chickens (EGK) and in turkeys (GB). In ducks, when the egg enters the uterus, the embryo already has several closed cells in the middle and is usually at a dStage 2.

TABLE 1. Strain effects on fertility, embryonic mortality, and developmental abnormalities during the oviduct period of development and from fertilization to 24 h of incubation or 72 h of incubation in two strains of Pekin duck

	Oviductal eggs		Fertilization to 24 h Incubation		Fertilization to 72 h Incubation	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Parameters measured						
Total eggs	263	278	283	273	455	529
Fertile eggs	261	254	280	240	443	
% Fertility	99.2	91.4***	98.9	87.9***	97.4	78.4***
Dead embryos	4	14	1	14	8	18
% Embryo mortality	1.5	5.5*	0.4	5.8**	1.8	4.3*
Abnormal embryos	1	6	2	3	21	18
% Abnormal embryos	0.4	2.4	0.7	1.3	4.7	4.3

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Area Pellucida and Hypoblast Formation

It appears that area pellucida and hypoblast formation in the duck, chicken, and turkey are similar. Temporal differences are observed relative to oviposition, and the onset of incubation and a well-defined Koller's sickle is observed only in the chicken embryo. Notwithstanding, area pellucida formation appears to be accomplished by cell shedding from the ventral side of the embryo. This phenomenon starts in the posterior part of the embryo (except for the most peripheral zone, which forms the future area opaca) and extends progressively towards the anterior end. Cell shedding is an ambiguous term affording no suggestion of the true biological mechanism of area pellucida formation. We are currently examining the role of apoptosis in area pellucida formation.

The first evidence of hypoblast formation (dStage 10) is not as distinct in the duck as it is in the chicken or turkey. In most duck embryos, polyingressing cells derived from the epiblast and presumably destined to be incorporated into the hypoblast are distributed uniformly over the ventral face of the area pellucida. Polyingressing cells are not observed in the marginal zone. At this stage the duck embryo is nearly symmetrical achieving a more polarized appearance later in development. A Koller's sickle was not detected in the duck embryo. However, a more diffuse arc of cells was observed adjacent to the posterior marginal zone of the area pellucida. The role of Koller's sickle and posterior marginal zone of the area pellucida in early embryonic development remains a contentious subject (Stern, 1990; Eyal-Giladi, 1991).

Although the morphological events characterizing the first 72 h of incubation in chickens and ducks are similar, the temporal rate of development between chicken, turkey, quail, and duck differ. These are compared and summarized in Table 2. Embryos of the four avian species are at the same developmental stage (around Stage 3) up to 9 h after the previous oviposition. After Stage 3, the speed of development between species diverges, with a faster rate of development in the species with a shorter incubation period. At oviposition, the quail embryo is more advanced (Stage 11) than the chicken embryo (Stage 10). Pekin duck and turkey embryos are developmentally less advanced. Interestingly, notwithstanding equal incubation length (28 d), the Pekin duck embryo is slightly more advanced at egg laying than is the embryo of the turkey (Stage 8 vs. dStage 7). Throughout the first 72 h of incubation, the Pekin duck embryo is less advanced than the chicken embryo at the same age.

The chronology of embryonic development of the Pekin duck described in the present study was shown to be equivalent to the observations of Chen (1932): primitive streak emergence at 14 to 15 h of incubation, head process emergence at 27 to 28 h, head-fold emergence between 30 to 33 h, and the appearance of the first somite between 33 to 36 h. Furthermore, no significant morphological differences could be observed between fertilization and 72 h incubation in the two strains of Pekin duck examined here. Thus, hatchability differences between the strains observed herein and in the commercial hatchery cannot be attributed to a delayed rate of Strain B embryos compared to Strain A embryos. Rather, hatchability differences seem to be due to increased infertility and increased individual development failures in Strain B.

Discussion addressing the basis of early embryonic mortality is generally focused in three areas. Management and environmental factors, genetic and chromosomal abnormalities (trisomy, aneuploidy, haplo-diploid chimerism), and the expression of biochemical factors or post-translation gene products (a protein deficiency, excess, inactive forms, hormone isoforms), or some combination or interaction of the three areas may be the basis of higher rates of developmental failures in particular strains. In this study, management and environmental factors are unlikely candidates to explain the fertility and hatchability differences observed. And until cytogenetic analyses are performed, one can only speculate as to the biological basis of the observed differences in fertility and hatchability between the Pekin strains examined herein.

Developmental differences between embryos from freshly laid eggs (dStage 8) and from unincubated stored eggs (dStage 10) are obvious and points to the need for good egg management from oviposition to when the egg is incubated. In his review article, Meijerhof (1992) high-

TABLE 2. Egg position, eggshell status, and embryo developmental stage in the oviductal period in two strains of Pekin duck compared to the chicken, turkey, and quail[1]

Hours after previous oviposition	Egg position in the oviduct	Eggshell status	Developmental stage[1]				
			Duck				
			Strain A	Strain B	Chicken	Turkey	Quail
3	Magnum	No membrane	1	1	. . .	. . .	1
6	Isthmus	Transparent to translucent shell membrane	1	2	1	1	1
9	Uterus	Opaque shell membrane	3	3	3	3	2-3
12	Uterus	Beginning of calcification	4	5	4-5	3	5
15	Uterus	Slightly hard but very crumbly shell	6	6	5-6	4-5	8
18	Uterus	Hard but easily breakable shell	6-7	6	7	5-6	8-9
21	Uterus	Hard and strong shell	7	7	8	6	9-10
24	Uterus	Complete shell	8	7	9	6	10-11
Oviposition	—	Complete shell	8	8	10	7	11

[1]Based on chicken embryo stages described by Eyal-Giladi and Kochav (1976); turkey embryo stages described by Gupta and Bakst (1993); and quail embryo stages described by Stepinski and Olsanska (1983).

lighted that the interval between oviposition and egg collection, egg storage conditions, and the prewarming treatments prior to incubation have a considerable effect on hatchability. Therefore, knowledge of an "optimum" developmental stage of the embryo at setting should be of significant interest to the commercial duck industry.

Egg Characteristics Usable as Indicators of Embryonic Development

During the oviductal phase, the combined thickness of the shell membrane and shell proper is highly correlated to the developmental stage of the embryo. As in quail (Stepinska and Olszanska, 1983) and turkey (Gupta and Bakst, 1993), shell thickness is an indicator of embryonic development but only during the oviductal phase.

In Experiment 2, Strain A eggshells were thicker than the Strain B eggshells, whereas in Experiment 3 the opposite was observed. Eggshell thickness in Strain B was not significantly different between the two experiments. The highly significant increase of the eggshell thickness in Strain A between Experiment 2 and Experiment 3 has no obvious explanation. It may be attributed to the changes in environment among experiments: Experiment 2 was performed in winter and Experiment 3 was performed in summer. Strain A may be physiologically sensitive to temperature and humidity variations, which might have modified its calcium metabolism and caused a higher mobilization and deposit on the shell in summer. Strain B may be less sensitive to these variations.

During the first 72 h of incubation, the variables related to the embryonic development are correlated to the egg weight loss during incubation. Under the conditions of this study, this easily measured parameter gives a good estimation of the developmental stage of the embryos. However, egg handling and manipulation (storage, prewarming) before setting may influence the egg weight as well as its inner characteristics. Thus, an egg weight loss curve obtained with unstored eggs may not be suitable for stored eggs.

The fertility of a flock of broiler hens can be predicted by the analysis of the PL of a few dozen eggs (Wishart, 1995; Wishart and Staines, 1995). If the method can be applied to ducks, PL-sperm hole counting could give accurate information on the reproductive ability of the breeder flocks throughout the laying cycle, even when natural mating is used. This procedure is difficult to perform with duck eggs for two reasons: the albumen tenaciously adheres to the PL thereby obstructing visualization of the sperm hole, and the sperm holes are noticeably smaller in diameter and the germinal disc imprint more difficult to discern than in the turkey or the chicken (M. Bakst, personal observations).

To conclude, there are practical applications of the present findings to the commercial duck industry and to poultry scientists investigating aspects of fertility and hatchability in the duck. The present staging procedure describing the sequential development of the duck embryo from initial cleavage through 72 h of incubation should be used to objectively assess the impact of egg handling and storage procedures and incubation conditions on hatchability.

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